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16. ABSTRACT

This is a review of literature regarding the fate and consequences of tritium in plants and soils. The kinetics of tritium in plants and soils was reviewed in light of water potential terminology, and some original research data are enclosed to illustrate specific concepts. The review cites 70 articles.

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TRITIUM IN PLANTS AND SOIL

by

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INTRODUCTION

The release and fate of radioactive elements in the environment resulting from nuclear weapons testing and the activities associated with the rapidly expanding nuclear power industry are major public concerns. Tritium, the only radioactive isotope of hydrogen, constitutes a significant portion of the residual radioactivity being released during these activities. Tritium is also a naturally occurring radionuclide which originates in the atmosphere from the action of certain components of the cosmic ray flux on the nuclei of nitrogen and oxygen in air; however, this results in a natural equilibrium concentration of only 16-35 picocuries per liter (pCi/l) in the hydrosphere. Weaver et al. (1969) attributed about 4% of the total estimated world tritium burden of 1.7×10^9 curies to the natural equilibrium level; the remainder was primarily ascribed to the atmospheric testing of nuclear weapons. Since tritium decays with a half-life of 12.35 years, large quantities of tritium produced by the atmospheric nuclear weapons prior to the 1963 test ban treaty are slowly decaying and the resultant concentrations in world waters and in the atmosphere are decreasing. Except for tests by nations not adhering to the treaty, the primary source of tritium currently being released is the nuclear power industry. Cowser et al. (1966) predicted that the world tritium burden would continue to decrease until about 1995, at which time it would start to increase due to the expanding production and release by nuclear power related activities.

Tritium concentrations presently observed in the drinking water of the United States vary from below 200 pCi/l (the detection limits of the Environmental Protection Agency surveillance network) to 3,100 pCi/l (Office of Radiation Programs 1974). Localized variations are generally associated with either nuclear activity or topography. For instance, Mullins and Stein (1972) showed that higher levels occurred in the mountainous regions of the western United States than at lower elevations.

Tritium is released to the environment primarily as tritiated water (HTO) and molecular tritium (HT, or T_2). Once released, tritiated water is rapidly diluted by the large volumes of water flowing in streams, rivers, and oceans, and, when released via stacks, by dispersion in air. It has been assumed that when elemental tritium is released it is diluted by the whole atmosphere. However, there remains some uncertainty as to its eventual fate in the atmosphere

and to the relative significance of its contribution to environmental tritium pollution. The large dilution capacities of the hydrosphere and atmosphere reduce the current average tritium concentration to very low levels, posing only a potential threat for the distant future. Nevertheless, tritium can be a problem on the local level.

Considerable research on the impact of environmental tritium on man's existence has been reported and various aspects have been reviewed. Outstanding publications on environmental tritium include a review by Jacobs (1968) on the physical and chemical aspects; another publication by Elwood (1971) which extensively covers the pathways and rates of tritium movement, the forms of incorporation, and biotic turnover; and a collection of papers edited by Moghissi and Carter (1973), which is based primarily on a conference held in September 1971 in Las Vegas, Nevada, during which an effort was made to evaluate all important aspects of tritium.

This paper focuses on those aspects of tritium which are specifically important for an understanding of the role and behavior of this radionuclide in the plant-soil-water system. Plant and soil literature was reviewed in light of water potential terminology and wherever possible, an effort was made to relate the studies carried out by agriculturists to those conducted by radiation biologists. In addition we have supplemented the review with the results of original research where a particular point could be explained more clearly by examples and where new data were required to demonstrate a concept.

CONCLUSIONS

Present knowledge of tritium movement in the environment is clouded by several contradictions and by incomplete understanding. The contradictions primarily arise in field studies where fractionations are reported for the exclusion or accumulation of tritium in plant materials. From controlled studies, it has been concluded that fractionations do occur, however, the maximum fractionation appears to be about 20% between the free water and the organic constituents of plants. Thus, even at its maximum, fractionation and exchange between protium and tritium may account for some important errors in analysis of plants and soils. These errors generally occur in sampling and sample preparation and may result in significant errors in interpretation of data.

Plants make up a large portion of the human diet and therefore serve as an important vector of tritium to man. Elevated tritium concentrations in crop plants will primarily be the result of contamination of soil with tritiated water, but the possibility of plant contamination from gaseous releases is also important. The impact of elemental tritium as a pollutant is incompletely understood. Recent data showing a rapid conversion of elemental tritium (HT) to tritiated water (HTO) in the environment of plants and soils suggest that further study is needed to evaluate the impact of this form of tritium as a pollutant.

CHEMISTRY AND PHYSICS OF TRITIUM AND TRITIATED WATER

The three hydrogen isotopes protium (P)*, deuterium (D), and tritium (T), have atomic weights of 1, 2, and 3, respectively. Each has one proton and one electron, and thus all have similar chemical properties, but since each has a different mass, the free energies of the isotopes vary and the corresponding reaction rates vary proportionately. The melting and boiling points of the hydrogen isotopes are listed in Table 1. The differences are accounted for by the amount of molecular free energy each isotope possesses. This decreases with increasing mass. Since the mass of HT is the same as that of D_2, their boiling and melting points, and other energy-related phenomena should be approximately equal, although these data are not reported.

TABLE 1. MELTING AND BOILING POINTS OF THE HYDROGEN ISOTOPES[a]

	Melting Point °K	Boiling Point °K
H_2	13.96	20.39
HD	16.60	22.13
D_2	18.73	23.67
T_2	———	24.9[b]

(a) Adapted from Holleman-Wiberg (1971).

(b) Friedman, White, and Johnston (1951).

Besides its difference in atomic weight, tritium loses a beta particle in the process of its decay to helium. All biological or physical differences between tritium and protium are based on either the mass difference, the effect of beta emission, or the different chemical properties of the tritium decay product. Deuterium, on the other hand, is nonradioactive

*Protium is rarely referred to as such and is generally referred to by the symbol for hydrogen (H). Alternate nomenclature follows the normal pattern for designating isotopes, i.e., ^{1}H, ^{2}H, ^{3}H.

and differs from protium only in mass. Deuterium studies have therefore been useful in interpreting the results of experiments with tritium. They have allowed discrimination between mass effects and radiation effects to be elucidated.

Eisenberg and Kauzmann (1969) reported the bond angles and lengths for D_2O, H_2O and HDO as being very near equal and pointed out that this was consistent with the Born-Oppenheimer approximation, which predicts that the electronic structures of molecules are independent of the masses of their nuclei. Electronic and spatial characteristics largely determine the chemical properties of an atom or molecule, therefore, it would be expected that all reactions involving hydrogen should be similar to those involving deuterium or tritium. Reaction rates are governed by molecular free energies which in turn are functions of molecular masses; thus although reactions are similar, slower reaction rates generally occur with the heavier isotopes.

The properties of water which reflect average free energies of solutions are termed colligative properties. These include boiling and freezing temperatures and vapor and osmotic pressures. The average free energy of a solution is primarily determined by dissolved solutes, matrix properties of structural materials, temperatures, and pressure. Materials dissolved in water and structural material in contact with water are termed hydrated, that is, surrounded by layers of water molecules. The forces which cause this hydration restrict the free movement of these water molecules and, as a consequence, the average free energy of water in a system is reduced. This change in average molecular free energy causes changes in the physical properties of water. A treatise on how various factors affect the average free energy, or the water potential in plants, was written by Slatyer (1967) and is valuable in interpreting the kinetics of tritiated water. In deuterated and tritiated water, the effect of the HDO and HTO molecules on the average free energy is similar to that of a solute, although for a different reason. Because of increased mass, the free energy of heavy water molecules is smaller than that of light water molecules. Consequently, the addition of heavy water to light water will result in an average free energy decrease of the solution. This decrease is obviously proportional to the concentration of the heavy water molecules. Physical properties reported in the literature for some properties of water altered by the presence of different hydrogen isotopes are listed in Table 2.

In addition to other factors the evaporation rate of a body of water is directly proportional to the average free energy of the water molecules in the liquid phase; lowering its average free energy by addition of a solute or deuterated or tritiated water molecules results in a lower evaporation rate. Evaporation rates are measured on the basis of molecular

TABLE 2. BOILING POINTS, TRIPLE POINT TEMPERATURES AND PRESSURES, AND MAXIMUM DENSITIES

	Boiling Point, ^{o}C[a]	Triple Point Temp., ^{o}C[a]	Triple Point Pressure, cm Hg[a]	Max. Density, g/cm^3 [b]	Temp. of Max. Density ^{o}C[b]
H_2O	100.00	0.010	0.458	0.999973	3.98
D_2O	101.42	3.82	0.502	1.10589	11.2
T_2O	101.51	4.49	0.492	1.2150	13.4

[a] W. M. Jones (1968).

[b] D. G. Jacobs (1968).

averages and are commonly expressed in milliliters per minute (ml/min); however, evaporation does not proceed on an average basis but occurs on an individual molecular basis. The probability of any molecule changing phases depends on its proximity to a liquid-gas interface and on its vibrational activity. In a mixture of heavy and light water molecules, the heavy molecules have a smaller free energy than the lighter ones and consequently their relative frequency of evaporation is lower. The average or overall effect of heavy water molecules on a solution is therefore the same as the addition of a solute, that is, the overall water evaporation rate is decreased. This occurs in direct proportion to the relative abundance of the heavy molecules. But because the heavy molecule does not affect the movement of the lighter ones, the lighter molecules evaporate faster resulting in an enrichment of the heavier molecules in the liquid phase. This type of isotopic fractionation can be described by the fractionation ratio. (FR), which is defined as

$$FR = \frac{C_2}{C_1}$$

where C_1 = concentration of heavy isotope in the primary fraction (i.e., tritium concentration in the liquid phase).

C_2 = concentration of heavy isotopes in the secondary fraction (i.e., tritium concentration in the vapor phase).

For HTO the tritium concentration is directly proportional to the specific radioactivity in curies per milliliter (Ci/ml) which can be used as C_1 and C_2 to calculate the FR. Since this term is the ratio of two concentrations it is unitless and can be

expressed as a decimal fraction or a percent. It is emphasized that the fractionation ratio describes the degree of fractionation and is independent of the original solute concentration (e.g., HTO), whereas, the overall rate of evaporation is directly dependent on the concentration of the heavy isotope. Under environmental or typical experimental conditions, the HTO concentration is so low that its influence on the evaporation rate and on all other colligative properties of the solution can be neglected. This has not always been the case in deuterium studies, and the lack or consideration of changes in water potential may have resulted in a misinterpretation of experimental results.

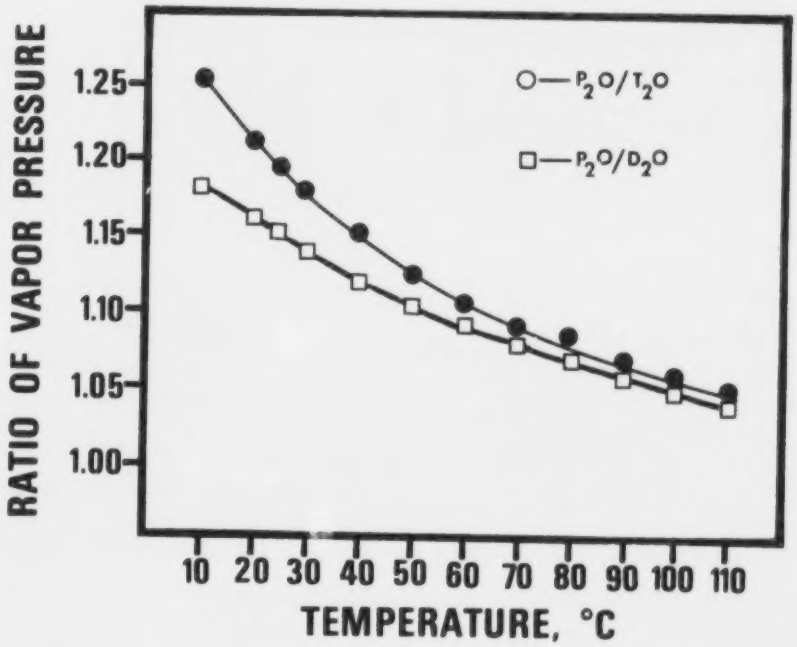

FIGURE 1. Ratio of Vapor Pressures of P_2O/T_2O and P_2O/D_2O. Jones (1968) as presented by Moghissi et al. (1973a).

Moghissi et al. (1973a) pointed out that temperature changes affect the vapor pressures of T_2O, D_2O, and H_2O to a different degree. The ratios of vapor pressure of H_2O/T_2O and H_2O/D_2O are presented in Figure 1. Both ratios decrease with increasing temperature. There is also corresponding decrease in the fractionation ratios with increasing temperatures. In addition, the water potential of heavy water is similarly affected by temperature. In Table 3 are shown the calculated water potentials of pure D_2O at various temperatures. Since

the water potential of pure free H_2O at a given temperature is defined as zero, it is clear that this isotopic difference in water potentials correspondingly decreases with increasing temperature.

TABLE 3. WATER POTENTIAL OF D_2O AT VARIOUS TEMPERATURES

Temperature ^{o}C	10	20	30	40	50	60
Water Potential* in Atmospheres	-193	-181	-166	-151	-137	-124

*Water potential calculated from the relationship

$$\Psi_w = \frac{RT \ln e/e_o}{\overline{V}_w}$$

where R = 0.082055 liter-atm. deg^{-1} $mole^{-1}$, T = ^{o}K, e = vapor pressure, e_o = vapor pressure of pure free water at T, $\overline{V}$ = partial molal volume.

TRITIATED WATER IN SOIL

Tritiated water applied to or accidentally deposited on a soil surface moves downward as a pulse. Originally there is a sharp boundary between contaminated and uncontaminated layers but this is readily blurred by exchange with soil water. Even in dry soils exchange of tritium with hydrogen on various particle surfaces results in a gradient at the forward edge. New water added to the soil surface pushes the old water downward creating a contaminated water lense (Figure 2). The rate and extent of downward movement depend mainly on the structure, texture, and water content of the soil. Agricultural research has been conducted for many years to describe the influence of soil texture and structure on the rate of water movement and on its availability to plants. This vast reservoir of knowledge can be put to good use in the study of the behavior of tritiated water in soil. Unfortunately, agriculturists and radiobiologists have different requirements and consequently, the data generally gathered by each discipline are not wholly usable by the other. For instance, agriculturists have been primarily concerned with soil water *in toto* and have had little need to emphasize the movement patterns of a particular pulse. However, the study of tritium movement in soil involves just that because only one portion of the water is of interest. Discussions of soil-water content and soil-water relationships are nevertheless valuable for tritium studies. These discussions can be found in many general texts, such as that by Black (1968), and in books directed specifically at the soil-water relationships, e.g., Nielsen et al. (1972).

Water in soil can be regarded as being segmented into different compartments, e.g., free water, capillary water, etc. The vertical movement of a lense of tritiated water, and the broadening which occurs with time, depend on a complicated relationship between environmental conditions and various water compartments. Each compartment has its special set of characteristics which determine water movement through and water behavior in it. In the simplest case, unobstructed water percolation is possible primarily through the soil free space. Movement rates of this "free water" are governed by soil texture and structure. In many soils, free water is also distributed throughout the soil by passage through fractures, holes caused by insects, worms, reptiles, and mammal activities, and through lumens created by decayed plant or animal tissues. The rapid dispersion of tritiated water through voids and irregularities in the soil structure is often responsible for great and inconsistent variations

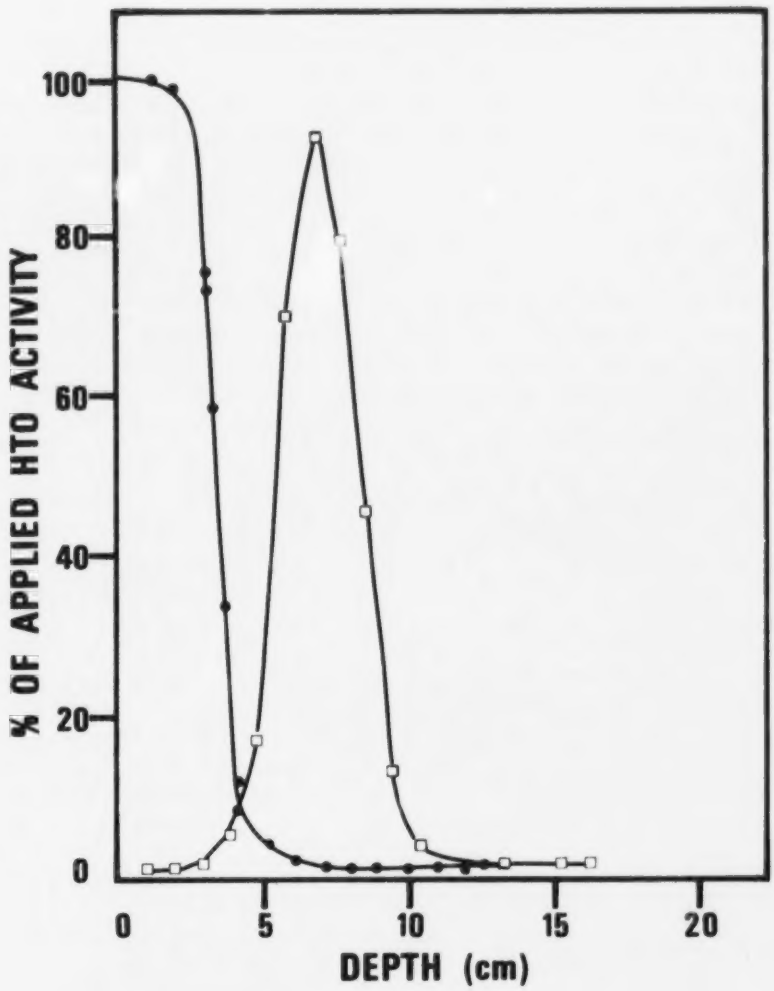

FIGURE 2. Downward movement of HTO in soil. Dilution of HTO at the wetting front (●——●) of an oven-dried soil is caused by exchange of tritium with protium in colloidal water and protium on the soil particles. After adding an equivalent amount of water to the labeled pulse, tritiated water lense is rather narrow (□——□). Diffusion causes this lense to become wider with time.

in a contamination depth profile. Investigations regarding this fast irregular dispersion of tritiated water have been conducted by various authors: Dixon and Peterson (1971), Jordan et al. (1971), and Sasscer et al. (1973). A correction for the water movement model of Sasscer et al. (1971) was derived by Jordan et al. (1974).

Water held as a thin layer on the surface of soil particles and in capillaries formed between soil particles and in decaying organic material is termed capillary water and, as such, constitutes a discrete compartment. Capillary water can move via mass flow whenever a water potential difference occurs in the capillary system as well as via molecular diffusion. Both modes of movement cause a broadening of the contaminated water lense. Molecular diffusion is non-directional and its extent depends primarily on the mean free path length and on the temperature. Most studies on the diffusive movement of water in soil systems have focused on vertical movements, since horizontal diffusion within a uniformly contaminated area is of no net consequence in terms of water availability. Yet horizontal movement may also be responsible for loss of tritium from a contaminated site to uncontaminated areas.

The sites of exchangeable cations on clay and organic matter can be classified as a separate compartment for the residence of tritium. Movement rates into and out of this compartment depend on the number of exchange sites, on the kinds and numbers of cations present, and on the soil water content. The exchange of tritium in the free and capillary water compartments with the cation exchange compartment is relatively rapid; therefore, exchange reactions are usually not important for the distribution pattern of tritium in soil. However, under a combination of certain conditions, e.g., when the tritium lense resides in the root zone, the soil is relatively dry and transpiration demands are high, this compartment can become significant. When Jordan et al. (1974) tested a water movement model, corrections based on tritium exchange with this compartment were required to fit their experimental data to the theoretical model. This was especially apparent towards the end of the growing season.

Tritium may also become an integral part of the clay mycell and reside in the form of hydroxyl groups attached mainly to aluminum and magnesium atoms. These hydroxyl groups exchange slowly with water from other soil compartments. This slow exchange rate renders the effect of this compartment on movement of tritiated water through soil and its availability to plants rather unimportant. Exchange in this compartment may be a useful tool in clay minerology and in geophysical studies since it allows the identification of specific reactions and may be useful in identifying certain time-dependent interactions. There have been studies regarding the possibility of isotopic fractionation in the exchange of tritium and

deuterium with the clay water compartment. Stewart (1967) reported fractionation ratios as high as 3:1 for a type of kaolinite clay, but in more recent studies he was unable to repeat these observations (Stewart, 1973) and instead showed the highest ratios were near one. Additional discussion of exchange in this compartment can be found in clay minerology texts and specifically in articles by Rabinowitz et al. (1973), Helevy (1964), Corey (1968), and Coleman and McAuliffe (1955).

Soils contain varying amounts of organic material (humus), most of which is derived from cellulose via degradation processes. This organic material contains exchangeable hydrogen which is bound to oxygen (and to a much smaller degree, to nitrogen). The amount of exchangeable hydrogen in this compartment depends on the amount of organic material present, on its degradation mode and on soil pH. These hydrogens exchange rapidly with HTO, thus slowing the downward movement of a tritiated water lense. However, soils high in organic material generally occur in areas where precipitation and percolation rates are high. Although in principle the interaction of tritiated water with the soil organics compartment has an effect on tritium movement in soil, it is relatively small in most natural systems, due either to low organic content or high percolation rates. However, additional information is desirable to more fully clarify the significance of this soil compartment to tritium movement in soil.

In addition to the effects of the various soil compartments on movement of tritiated water in soils, other factors can have a marked influence. When tritiated water is applied to a soil surface, a rapid initial evaporation loss will occur under most circumstances. The extent of this loss depends on a number of factors such as climatic parameters, soil composition and texture, kind and extent of plant cover, etc.

Water loss via plant root absorption and transpiration is of great importance in most terrestrial ecosystems. Plants with a shallow root system such as many vegetables and cereals absorb all of their water from the upper few centimeters of the soil whereas other plants with tap roots may absorb water at depths of 10 to 15 meters. Thus, movement of a water lense to a meter below the surface may make this body of water unavailable to some species, but optimize absorption for others. Diversity in rooting patterns also results from differences in plant age, vigor, soil type, climate, and on the number and kinds of plants competing for space, water, and nutrients. This great diversity makes it difficult to predict the importance of plant transpiration on the fate of a pulse of tritiated water. Although generalizations are possible and valuable each situation must be evaluated individually.

The speed with which water moves from soil to plants was illustrated in an experiment conducted by Woods and O'Neal (1965). Tritiated water added to soil near an oak tree was detected in the leaves after only four hours. We have observed it in alfalfa and lettuce plants within minutes of application to the soil. The residence time of water in plants is generally short in comparison to its residence time in soil, notable exceptions being certain desert plants (e.g., cacti). This is a result of the large water-holding capacity of soil combined with the large soil mass. Thus the residence time of tritium in a soil system largely determines the exposure time, and consequently the effect, on the rest of the ecological system. The relatively short residence of tritium in mesophytic plants growing on tritium contaminated soil is extended by the longer residence of tritium in the soil. Root zones contain tritium for varying lengths of time depending primarily upon rainfall, soil characteristics, and on environmental parameters which influence movement in and out of the various soil-water compartments. There are several reports of particular value which should be studied to obtain a broader understanding of the interactions between soil and plants growing under different climatic conditions. Among these are reports by Koranda et al. (1967, 1968, 1969), who studied tritium movement in desert soils following the Sedan detonation which dispersed approximately one million curies of tritium into about six million tons of earth. Koranda and Martin (1973) reported on the movement of a pulse of tritiated water in an irrigated corn field. Jordan et al. (1970) report on the movement of tritiated water in a tropical rain forest and in (1974) on its movement in a grassland community.

TRITIUM IN PLANTS

ROUTES OF ENTRY

Water enters plants primarily through the roots. Plant exposure to tritium therefore depends mainly on the concentration of HTO in the soil and the position of the contaminated water lense relative to the plant roots. Under some conditions the foliar absorption of tritium may also become important. Of particular concern are plants growing in areas with aerial levels of tritiated water vapor or elemental tritium gas (HT), such as may exist in the vicinity of some nuclear facilities.

The exchange and incorporation of liquid HTO into plant leaves was demonstrated by Vaadia and Waisel (1963). They exposed the leaves of Allepo pine (Pinus halepensis) and the common sunflower (Helianthus annua) to tritiated water for various periods and showed that, under conditions of no transpirational water loss, a rapid accumulation of HTO occurred in both species. Transpiring plants, in an environment of tritiated water vapor, accumulate tritium at a much slower rate (Koranda and Martin 1973), (Cline 1953), and (Aronoff and Choi 1963).

The results of an experiment designed to measure foliar absorption of tritiated water by leaves and its subsequent translocation to the roots are shown in Figure 3. Alfalfa plants growing in sealed hydroponic containers were introduced into a growth chamber which was maintained at a constant HTO vapor concentration of 110 nanocuries per milliliter. Lights remained on continuously during the first 72 hours but were turned off the entire fourth day. Samples of the root nutrient solutions were collected periodically during the experiment and analyzed for tritium. Every 24 hours distilled water was added to each hydroponic container to replenish the water transpired by the plants. These dilutions account for the observed minima of the tritium solutions as illustrated in Figure 3. At the conclusion of the first portion of this experiment (lights on) the tritium concentrations in the nutrient solutions had increased from zero to about 40 picocuries per milliliter (pCi/ml) (0.07% of the steady state atmospheric level). On the fourth day of the experiment the lights were deliberately left off and the transpiration rate decreased from about 4×10^{-3} milliliters per minute per gram (ml/min/g) of fresh leaf tissue to about one-tenth that value. In the light (high transpiration rates) basipetal translocation of water vapor absorbed by the leaves was accounted

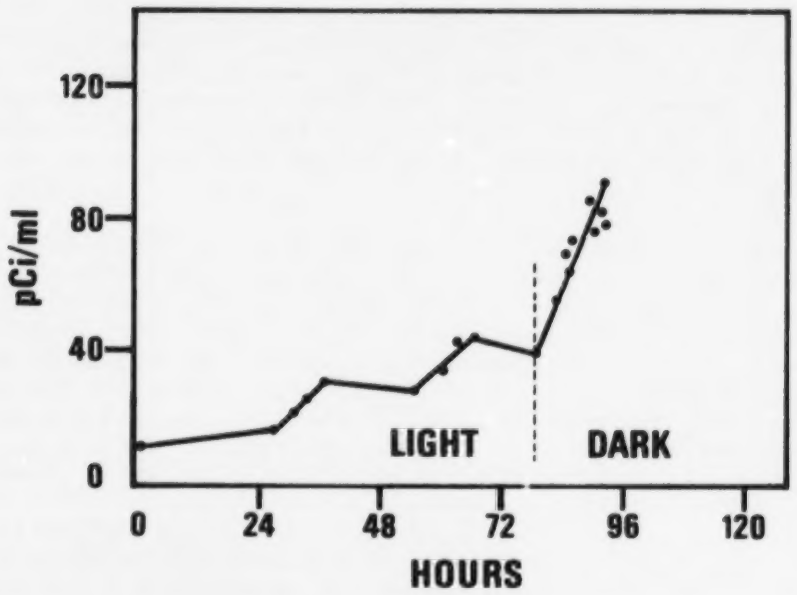

FIGURE 3. Tritium concentration in the hydroponic solution of alfalfa plants. The route of tritium contamination to the solution was by foliar absorption of HTO vapor.

for by a net movement of 0.8 milliliters per day (ml/day). Obviously, foliar absorption and water loss from roots were much greater than this value but because of water mixing between phloem and xylem the net transport was small. When the lights were out and transpiration rate decreased, the rate of basipetal movement was increased to 2.4 ml/day. Under these conditions the foliar absorption rate was decreased because of increased stomatal resistance. But because of decreased dilution coincident with slower rates of water movement in the xylem, the resultant basipetal movement was increased by a factor of 3. At the termination of the 24-hour dark period, the extractable free water of the roots and leaves was found to contain 521 and 39,431 pCi/ml, respectively. These values correspond to 0.5% and 35% of the tritium concentration present in the vapor phase. The concentration gradient from the leaves to the roots is thought to be due to dilution via exchange with water moving upward in the xylem. In the dark, when less water was moving upwards, less dilution occurred, and as a consequence, an increased rate of HTO accumulation in the hydroponic solutions was observed. It is evident from these data that even under conditions of high relative humidity (85%), moderate transpiration rates, and continuous HTO vapor exposure, plants do not reach an equilibrium with the HTO in the atmosphere, and contamination of the roots and soil via foliar absorption would be very slow. It is also clear that under conditions of low net transpirational

water loss, that the leaves do accumulate tritium from contaminated water vapor. Since the leaf water is used as a photosynthetic substrate the photosynthate will naturally be contaminated to a level dependent on the leaf concentration. As pointed out, this may be determined by the soil water or water vapor depending on the environmental conditions and the type of plant involved.

Under most conditions, transpiring plants which are contaminated with HTO (regardless of the contamination route) rapidly lose tritium from the free water in the tissue, as was demonstrated by Koranda and Martin (1973). They found that both woody and herbaceous plants which had absorbed HTO from the gas phase lost tritium quickly with the residence half-times varying between 20 and 70 minutes, depending on differences in water turnover rates. The plant with the largest transpiration rate obviously had the shortest residence half-time.

Recent experimentation by Kline and Steward (1974) regarding foliar tritium uptake and loss from atmospheric HTO have yielded some interesting rates. Their conclusion aptly summarizes the importance of the foliar contamination of transpiring plants.

> "In the event of accidental HTO release to the atmosphere near ground, rapid contamination of vegetation could be expected. When the atmospheric source is dissipated, rapid decontamination could also be expected during periods of normal plant transpiration but not at night or other times when transpiration flow is not taking place."

Under certain conditions, e.g., near nuclear facilities or in the vicinity of natural gas wells which had been stimulated by nuclear explosions, plants might be exposed to significant levels of HT or tritiated methane (CH_3T). McFarlane (1976) has shown that when plants were exposed to HT they rapidly incorporated tritium into the free water and the organic material of the plants. The significance of this observation and the rate of the conversion of HT to HTO are presently under investigation. Mason et al. (1971, 1973) determined in the field and in the laboratory that plants incorporate tritium into the plant free-water and into the organic phase when exposed to CH_3T. Their data indicate that in transpiring plants the incorporation of tritium from CH_3T into the leaf free-water reaches steady state in about 18 minutes. They concluded that the deposition velocity of tritiated methane was only about 4% of that of HTO vapor.

In summary, root absorption of HTO is the most important pathway of tritium entry into plants. Tritiated water vapor appears to be of consequence only if it occurs at very high

levels persistent over a long period of time in conjunction with high humidity which results in low or zero net transpiration, or if it condenses in the form of dew to become a component of the soil water. The foliar contamination of plants by CH_3T, although an observed fact, appears to be relatively unimportant because of the infrequence of release and the relatively low incorporation. The consequence of plant exposures to HT is not yet fully understood. It is known, however, that large quantities of HT are released during nuclear fuel reprocessing operations, and the foreseeable increase of nuclear facilities demands a through examination of the resulting increased exposure of plants to HT.

TRANSPORT IN PLANTS

Plant-water relations have been the subject of extensive investigations and elaborate mathematical models have been developed to describe both water stress and water movement in plants (Slatyer, 1967). Under mass flow conditions (i.e., transpirational water movement through the xylem) HTO moves at the same rate as H_2O. In diffusional flow, (i.e., evaporation or intracellular movement), HTO moves at a slower rate than H_2O due to its larger mass. As previously indicated, these differences are so slight and water movement in plants so dependent on mass flow, that diffusional differences are inconsequential and are generally considered unimportant in describing tritium movement in plants and soil. Therefore, the net movement of tritiated water can best be described by systems developed to identify and define water movement.

More than 99% of the water entering most plants moves from the root through the xylem system to the stems and leaves, where it is rapidly lost to the atmosphere via stomatal openings (Lieth, 1963). The residence time for water in plants varies between minutes and days, depending on parameters such as species, size, age, plant morphological features, soil water availability and atmospheric conditions. Also constant exchange with metabolic water causes dilution and a fairly rapid loss of HTO from plants which have had an acute exposure. Following imbibition and equilibration by soybean seedlings exposed to HTO, it was shown that the tritium depletion rate from the free water component of the seedlings was exponential. Twenty-four hours after tritiated water in the rooting zone was exchanged with H_2O, only 1% of the original tritium concentration remained in the plant. After 120 hours, less than 0.01% of the original concentration was present (Vig and McFarlane, 1975).

Investigations using HTO as a tracer in soil-plant studies have illustrated the rapid movement of water in these systems. For example, Woods and O'Neal (1965) observed tritium in oak leaves 4 hours after an addition of HTO was made to the soil. Lewis and Burgy (1964) also experimenting with oak

found tritium in the leaves after a soil application 55 feet from the base of the tree. In another investigation it has been observed that water movement occurs primarily in the outermost vessels of the xylem (Wray and Richardson, 1964).

ISOTOPE FRACTIONATION IN PLANTS

It was previously pointed out that there are no qualitative differences in the chemical and physical behavior of the three hydrogen isotopes. However, differences in the mass of protium, deuterium and tritium result in reaction rate differences which are dependent on the amount of free energy in the molecules containing them. Under identical conditions, heavier molecules have lower velocities and thus experience fewer collisions and also have lower vibrational energy and consequently experience a smaller chance of dissociation. Differences in rates result in fractionation. Significant fractionation of hydrogen isotopes in biological systems can occur in phase changes such as the evaporation of water and in chemical reactions like the synthesis of chemical compounds in plants and animals.

Since fractionation occurs during evaporation of H_2O/HTO mixtures, it may seem puzzling that there is no agreement in the data indicating fractionation in plant transpiration (Washburn and Smith, 1934; Zimmerman et al., 1967). It can be rationalized that, if fractionation occurs during transpiration (in the evaporation step), then the concentration of HTO would increase at and near the evaporative surface. This increasing concentration of HTO at the evaporative surface would cause a concomitant increase in the amount of HTO evaporated (which is proportional to the concentration) until a steady state is reached wherein the loss of tritium would be exactly equal to the supply of tritium. Under conditions of rapid water loss, the accumulation of HTO would be restricted to the evaporative surface and very little diffusion of enriched HTO into the surrounding leaf tissue would be expected. As a consequence, the overall enrichment of tritium would be very small. Under slow transpirative conditions, tritiated water enriched at the evaporative surface would be expected to diffuse into surrounding tissue, thus raising the overall tritium concentration in the leaves to a higher level. Diffusive movement away from the evaporative site and mass flow of tritium in the phloem may result in increased concentrations in the nontranspiring tissues. Consequently, the transpiration rate, not only determines the rate of buildup of HTO at the surface, but also the amount of HTO present in the leaves and other organs. This rationale has led to the hypothesis that fractionation of water may only be evident under certain conditions, i.e., low transpiration. Fractionation is not normally observed because even though evaporation is a diffusive phenomena (subject to fractionation), the supply of water to the evaporative site is by mass flow (no fractionation)

which entails a more rapid velocity of water molecules moving toward the evaporation site than the diffusive movement of HTO molecules moving in the opposite direction.

To test this theory, we grew alfalfa plants in hydroponic solutions containing HTO in closed environmental simulators. Only the humidity in the simulators was altered to vary the rate of transpiration. The plants were exposed to 8 hours of constant humidity before sampling to ensure that equilibrium had been obtained. The leaves were separated from the stems, and water was extracted from the respective tissues. The tritium concentration in the extracted water was determined by liquid scintillation counting. The results which are listed in Table 4 indicate a significantly higher tritium concentration in the water of the leaves collected under slow transpiration conditions. This slightly higher level is thought to be evident because of diffusion of HTO-enriched water away from the evaporative surfaces into the surrounding mesophyll tissue. Despite this fractionation at the evaporative surface, under most experimental or environmental conditions any fractionation occurring in the process of transpiration would be so small as to make its contribution to biological processes insignificant.

TABLE 4. TRITIUM CONCENTRATION IN FREE WATER OF ALFALFA STEMS AND LEAVES UNDER CONDITIONS OF SLOW AND RAPID TRANSPIRATION

nCi/ml

	SLOW TRANSPIRATION (High humidity > 70%)	RAPID TRANSPIRATION Low humidity > 25%)
Leaves	77.2 ± 0.6*	75.4 ± 0.5
Stems	75.4 ± 0.5	75.8 ± 0.5
Hydroponic Solution	75.0 ± 0.6	75.1 ± 0.5

* Significantly different from other means at the 0.05 level based on paired T tests.

± The 95% confidence interval

The thermodynamic basis for discrimination among the hydrogen isotopes in the synthesis of organic molecules was established by Urey and Rittenberg (1933) and expanded by Bigeleisen (1949) and Waterfield et al. (1968). The observations of fractionation of tritium between supply (free water) and incorporation in the organic molecules in plants were reviewed by Bruner (1973) and Weston (1973). These reviews list examples

of both concentration of and discrimination against tritium in organic incorporation. Since tritium is heavier than protium, it is difficult to imagine a condition where it would be incorporated at a faster rate than protium. In reactions such as the elimination of hydrogen from a particular site (e.g., in metabolic processes), the heavier isotopes are transferred at lower rates than hydrogen. As a result, an elevated tritium level could develop in certain fractions. Subsequent synthetic reactions based on these fractions as substrates could then lead to compounds with a higher tritium content than the bulk water supply.

Smith and Epstein (1970) demonstrated that all plants tested gave a similar fractionation ratio between protium and deuterium where the source of water was common to all species. They also showed that although very little discrimination against deuterium occurred at the cell membrane, the photosynthetic splitting and incorporation of the water fragments into organic compounds resulted in a fractionation ratio of 0.958, or in other words, there was a discrimination of 4.2%. An additional fractionation of about 9.2% occurred between carbohydrate and lipid. The overall fractionation from water to lipid ranged from about 13% to 17%. Fat synthesis was previously singled out by Bokhoven and Theeuwen (1956) as a process resulting in a considerable isotope effect. The same authors could also distinguish synthetic and fermented alcohol by the lower deuterium content of the latter due to the combined discrimination effects against deuterium in various metabolic processes.

Very high fractionations have been reported by some authors. For instance, Weinberger and Porter (1953) measured tritium incorporation in Chlorella pyrenoidosa and reported that the specific tritium activity in this aquatic plant species was only 47% of the specific activity of the water pool. Later, the same authors (1954) reported fractionation between various organic fractions. Their method employed extracting components using methanol and ether and isolating cells by repeated resuspensions in water. Unfortunately this method allowed ample opportunity for tritium to exchange with the hydrogen in the solvents. Consequently, fractionations which they reported are probably unrealistically high.

In an attempt to evaluate the extent of fractionation possible in plants, McFarlane (1976b) grew alfalfa plants in a sealed growth chamber. In these experiments great care was taken to assure that equilibrium conditions persisted throughout the growing period. It was shown in these experiments that a discrimination of 22% occurred against the incorporation of tritium into the total organic complex which made up the leaves and stems.

It is evident from the reported results that the extent of fractionation in plants and its importance as a possible means of concentrating or reducing tritium concentrations is small. Therefore, future research should be directed not to merely observe and document fractionation between water and plants, but rather to determine the sites and the extent of fractionation.

TRITIUM IN AQUATIC PLANTS

In aquatic systems the retention and turnover of tritium in vegetation depend on the kind and duration of exposure, which in turn depend on the type of insult (chronic or acute) and the nature of the water body. For instance, in a large turbulent river contaminated by a single pulse of tritium, rapid dilution will occur and incorporation of tritium into aquatic plants will be restricted to a relatively short period. Consequently, the exposed plants would have a very short accumulation period followed by a retention period which would depend primarily on the morphological characteristics of the plants. On the other hand, a continuous release of tritium into a lake or other relatively stable body with a slow water turnover rate would result in a steady tritium accumulation by plants in equilibrium with the water.

Most information dealing with tritium in aquatic systems focuses on incorporation into aquatic animals; only a few published reports are concerned with the uptake by aquatic plants. Weinberger and Porter (1954) reported discrimination against tritium incorporation in the biomass formation of Chlorella pyrenoidosa grown in tritiated water. However, these results should be regarded with caution, as explained previously. Cohen and Kneip (1973) determined the tritium concentrations in water, sediments, and aquatic plants downstream from a nuclear reactor. Their data showed that "In the aquatic environment, there is significant incorporation and retention of tritium in the bound state of bottom sediment and biota." Tritium concentrations when compared to the concentrations in the contaminated water were, on the average, 10 times as high in sediments, 4 times as high in fish, and 3 times as high in rooted aquatic plants. Their conclusion was that plants may derive much of their hydrogen from the organic sediments on the river bottom. Unfortunately, they did not account for the impact of changing ambient tritium concentrations during different periods of time or at different locations in the river.

On the Nevada Test Site, Typha and Potamogeton samples were collected in Haines Pond by Brown (1971). This pond was formed by water seepage originating near an underground nuclear detonation, and contains a relatively high amount of tritium. Analysis of the two plant species showed a higher tritium activity in the free water and organic bound fractions

of both species than in the pond water. Although it would be tempting to assign this difference to isotopic fractionation, no valid interpretation of these results can be made without more details of the history, the water turnover rate and the contamination source. It is this temptation to draw conclusions based on incomplete data that has particularly plagued aquatic-tritium studies. The authors can find no compelling evidence in the literature of any large tritium fractionation in aquatic systems. To the contrary, it appears clear that free water fractionation does not exist in aquatic plants. However, fractionation against organic incorporation in the range of 10% or 20% would be expected.

EFFECTS

The effects on plants of tritium incorporation can be caused by the mass difference, the beta particle emission, or the properties of the decay product, helium-3, which differ vastly from the properties of hydrogen.

Deuterium is the ideal tool to study the role of mass difference because it differs from protium and tritium only in mass and is not radioactive. As far back as 1937, deuterium was reported as retarding respiration in yeast (Taylor and Harvey, 1937). Germination of seeds and spores and the growth of Chlorella were shown to be inhibited in proportion to the concentration of D_2O in the culture solution by Crumley and Meyer (1950) and Moses et al. (1958). Weinberger and Porter (1954) observed Chlorella cell enlargement in the presence of D_2O, and additional evidence by Bennet et al. (1958) pointed out that this can occur in the absence of cell division. They concluded that D_2O was only inhibiting the cell division process. They also observed that cells could be acclimated to D_2O by gradually increasing its concentration in the culture medium. Blake et al. (1968) suggested that the effect of deuterium on embryo development was ameliorated by the presence of large hydrogen-containing food reserves in the seed. The same researchers (Crane et al. 1969) later separated the embryos from various seeds and showed that D_2O inhibited all species in the same manner.

Biological effects caused by deuterated water molecules are probably associated with a change in the average free energy of metabolic water. Calculations of water potentials from the vapor pressures reported by Jones (1968) give the values listed in Table 3. It is obvious that putting tissue into pure D_2O would cause a tremendous osmotic shock, i.e., at 25°C it would be comparable to immersing the tissue sample into a solution more concentrated than 7M NaCl. Since the water potential (Ψ_w) equals the sum of the component potentials, the potentials of mixtures of D_2O (or HTO) with water are proportional to the abundance of D_2O (or HTO);

$$\Psi_w = \Psi_1 a_1 + \Psi_2 a_2$$

where Ψ_1 and Ψ_2 represent the water potentials and a_1 and a_2 represent the fractional portion of the solution volume represented by H_2O and D_2O (or HTO), respectively.

When HTO is added to H_2O, it is clear that the water potential of the solution is

$$\Psi_w = \Psi \text{ HTO}^a$$

since by definition $\Psi_{H_2O} = 0$

Thus, when evaluating experiments conducted with high levels of D_2O (or HTO), considerable attention should be given to the water potential gradient imposed by the treatment. Unfortunately, this aspect has generally been overlooked. Available literature indicates that at concentrations of D_2O less than 1%, effects are not generally evident. This seems to support the idea expressed above and may mean that insults do not take place, or on the contrary may mean that our detection systems are not sensitive enough to observe their manifestations.

In contrast to most deuterium studies, the concentrations of tritium found in the environment, and in most experiments, are such a small fraction of the total water content that the contribution of tritium to altering the average molecular free energy is generally insignificant and can be ignored. For example, it can be shown by using the specific activity of tritium (Ci/g), Avogadro's number (molecules/mole), and the molecular weights of HTO and H_2O (g/mole) that a solution of 1 microcurie per milliliter (μCi/ml) (which is much higher than environmental levels, but representative of some experimental concentrations) contributes only one molecule per 1.83×10^{11} molecules of H_2O. Thus the significant effects of tritium on biological systems are probably not due to mass differences, but are rather due to the beta radiation resulting from decay and/or due to the decay product.

The effect of ionizing radiation (x-rays) on bean roots has been one of the classical demonstrations of radiation effects. Many outstanding works on this subject were authored by Gray, Thoday, Read, and Scholes in the British Journal of Radiology from 1942 to 1952. Their work showed that the apical meristem of the root was radiosensitive and was therefore a good system to study relative biological effectiveness. Spaulding et al. (1956) repeated Gray's techniques and compared the effects of x-rays to the damage found in plants exposed to beta radiation from HTO. They immersed bean roots in HTO and calculated the dose from diffusion time and the specific activity of the treatment solution. Exposure times were from 1 to 4 hours and no consideration was given to residual tritium which became part of the organic molecules. Their calculations showed a relative biological effectiveness of 1.0 + 0.06 according to the equation:

$$\text{RBE} = \frac{\text{effect of } \beta \text{ dose}}{\text{effect of x-ray dose}}$$

They concluded that in their system, beta radiation derived from the decay of tritium in the plant water had the same effect both quantitatively and qualitatively as did 175 kilovolt peak x-rays applied externally.

In the process of preparing tracers by exposing soybeans to carbon-14 labeled carbon dioxide and HTO, Chorney et al. (1965) observed effects on the growth rate and gross morphology of the soybean plants. Somatic aberrations caused by tritium resulted in characteristic bulbous enlargements at the nodes and below the terminal influorescence and the leaves were mottled just as in other radiation treatments. These observations were assigned to a calculated accumulated dose of 1,000 rads which resulted from growing the plants in culture solution containing 37.5 µCi/ml of tritium. The differences in growth rates between the treated plants and the controls were reported, but these data are of a questionable value since in the hermetically sealed growth chambers the carbon dioxide (CO_2) concentrations were allowed to fluctuate between 50 and 1,000 parts per million (ppm) while the control plants were grown in atmospheric levels of CO_2. Seeds from the plants exposed to tritium contained 17.6 µCi of tritium per gram and, when stored for 44 days and then germinated, a pigment abnormality was observed in the primary leaves.

The continued culturing (18 months) of alfalfa plants in a closed environmental simulator containing 300 nanocuries per milliliter (nCi/ml) of tritium as HTO revealed neither detectable morphological damage nor alteration of any physiological parameter of the plants (McFarlane 1975). However, using an extremely sensitive indicator of somatic alteration, Vig and McFarlane (1975) have shown genetic effects in soybean plants when the seeds were germinated in water containing as little as 10 nCi/ml of tritium.

When a beta particle leaves an atom, a resilient energy is imposed on the atom, called recoil energy. In the decay of ^{32}P this energy is sufficient to cause bond breakage. In tritium decay, the maximum recoil energy is too small to have much effect on chemical bonds (Woodward, 1970). The decay product of tritium is helium-3 which is a noble gas with vastly different chemical properties than its parent. The replacement of the newly formed helium with stable hydrogen has been thought to occur with sufficient ease to cause little or no effect. This logic has led to the assignment of all damage caused by tritium in biological systems, whether somatic or genetic, to the effect of the radiated 3 megaelectron volts beta particle. One experiment which argues against this rationale was conducted by Funk and Person (1969). They showed that decay of tritium in the 5 position of cytosine resulted in a specific mutation. This specificity argues in favor of assigning the effect to transmutation of tritium to helium which causes interruption of the coding sequence at a particular point. When helium is formed, it immediately

leaves the site formerly occupied by the tritium atom, and the atom formerly bound to the tritium atom becomes a free radical. Free radicals are usually highly reactive, unstable species which can stabilize by combining with another free radical or by intramolecular rearrangement. This is often accompanied by the elimination of an atom or molecular fragment, and the formation of a double bond in the parent molecule. Therefore, it should be remembered that the possibilities of damage other than direct radiation damage have largely been ruled out by hypothesis and not by test.

Radiation doses from environmental tritium are very low. The Federal Radiation Council cites 170 millirems per year as the recommended maximum dose from all sources except medical radiation and natural background for the human population. This conservative figure is designed to be below the dose which could cause any damage to man. Using a quality factor of 1.0, the recommended maximum dose from tritium by man would be 170 millirads if tritium were the only source of radiation. Near the Humboldt Bay Pacific Gas and Electric nuclear power reactor, plant samples were collected which contained up to 3.8 nanocuries per liter of tritium in the extractable plant water. If this were a chronic contamination level, the plant would have an absorbed dose of 0.00044 millirads per year. Compared to the 170 millirads suggested as safe for man, this seems to be an insignificant dose. Compared to the 1,000 rads used by Spaulding et al. (1956) in their experiments, even the highest doses which were observed in plants near that reactor were miniscule. This is not an endorsement of the hypothesis that no effects would occur, but, if present, it is probable that they would not be observed because of their infrequence.

PITFALLS

Many pitfalls await the investigator studying tritium in biological systems. Experimental errors are generally associated with the isotopic fractionation effect, and most commonly occur during the steps of sample collection, storage, and preparation for analyses. It has already been pointed out that the tritium bound to oxygen as in water, alcohols, organic acids, carbohydrates, etc., exchanges with hydrogen bound to oxygen. Therefore, if a tritium-containing plant sample is in contact with moist air at any time between the time of collection and the time of tritium analysis, sufficient exchange with atmospheric water vapor may occur to invalidate the analysis. This is even more critical when the samples are in contact with solvents which contain exchangeable hydrogen. Not only is the plant water affected but tritium atoms incorporated into organic molecules as hydroxyls are also readily exchanged (Lang and Mason, 1960). Under appropriate conditions this could amount to a tritium loss of up to 30% from the organic plant material.

Tritiated water is often collected from plant, animal, and soil samples by freeze-drying or other methods of vacuum extraction. The fractionation between HTO and H_2O occurring during this step under some conditions may account for large errors. Because the degree of fractionation is inversely proportional to the temperature of the evaporating solution, the error is larger when the sample is chilled or frozen. Even without intentional freezing, sample temperatures always decrease during vacuum vaporization unless the heat loss due to the heat of vaporization is compensated for by heating the sample. Figure 4 shows the temperature fluctuations of plant and soil samples during vacuum vaporization extraction at an ambient air temperature of approximately 25°C. Water in both kinds of samples quickly froze when the vacuum was applied (0.1 millimeters of mercury); then the temperature gradually increased until the samples were completely dry. The water was collected from these samples in 5-ml increments and each increment was analyzed for tritium content. Figure 5 shows the relationship between the tritium activity in each increment and the total amount of moisture removed. From these data, correction factors could be used to calculate the true tritium concentration of the samples. However, this procedure is risky when high accuracy is desired, since sample type and size, rate of water vaporization, and temperature all independently influence the degree of fractionation. Using a vacuum extraction system, the only way to assure an accurate analysis of extractable water appears to be by

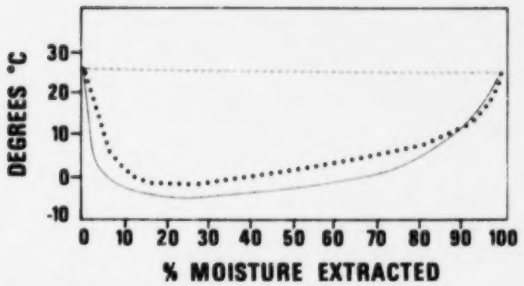

FIGURE 4. Temperature of a 100g soil sample (....) and a 150g plant sample (——) during vacuum extraction of water (McFarlane 1975c).

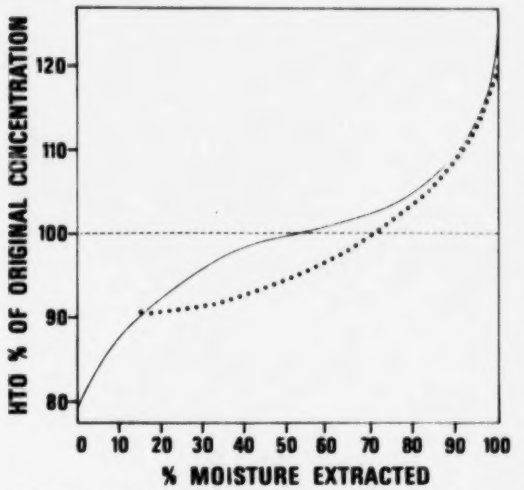

FIGURE 5. Tritium in water samples collected in 5ml increments from soil (....) and plant (——) samples by vacuum extraction (McFarlane 1975c).

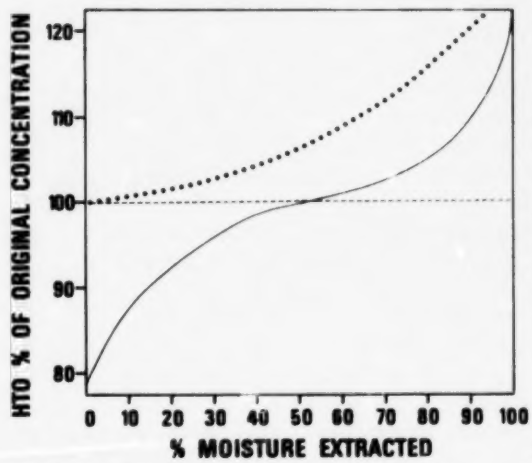

FIGURE 6. Fractionation of tritium in the water extracted from plants (——) and the calculated concentration remaining in the plant tissue (....) (McFarlane 1975c).

collecting 100% of the tissue water. Even this may result in errors, if organic tritium is part of the desired data. In Figure 6 it is shown that the tritium concentration of the free water remaining in the sample increases during the extraction up to the concentration of the last fraction. This may be 30% higher than the original tritium concentration in the free plant water. Since the tritium in water can readily exchange with up to 30% of the hydrogen atoms in the organic fraction, an artificial tritium enrichment (up to 9%) of the organic fraction could occur. Obviously the tritium content of the collected free plant water would be lowered by the amount transferred to the organic fraction.

A preferred method for critical work is based on the formation of an azeotropic mixture between the free sample water and a suitable organic solvent such as toluene or benzene. An excess of dry solvent is added to the sample, the azeotrope is recovered by distillation and the tritiated water separated from the solvent upon cooling. This method is reportedly less subject to isotopic fractionation (Cline, 1953; Moghissi et al. 1973b).

Another type of error which is also based on isotopic fractionation became apparent during experiments with HTO conducted in an environmental simulation chamber. The chamber humidity was controlled by a coil cooled to 10°C and the condensate dripping from the coil was analyzed for tritium as an indicator of the HTO concentration in the chamber air (Table 5, Column B). In a control experiment, air from the chamber was passed through a molecular sieve tap and the water extracted by azeotropic distillation; another air sample was passed through freeze traps and the water pooled for

analysis. Both control experiments gave identical results and are believed to be the true values for tritiated water vapor (Table 5, Column A). In a third part of the test, chamber air was passed through a tap water-cooled condenser (cooling water temperature approximately 20°C) for partial condensation followed by freeze traps which collected the remaining water vapor (Table 5, Column C and D). The data show that isotopic fractionation occurred whenever partial condensation occurred. Variations of the relative humidity in the chamber did not reveal any particular trend in the analytical results suggesting that the condenser temperature (10° or 20°C) were more important than the condensation rate. The difference between Column B and C is qualitatively in line with the expected temperature effect on the vapor pressure ratio of H_2O/T_2O as illustrated in Figure 1.

TABLE 5. TRITIATED WATER VAPOR COLLECTED BY DIFFERENT METHODS EXPRESSED AS PERCENT OF THE CONTROL VALUE

Relative Humidity	Control (A)	Cold Water Condenser Humidity Control Equipment (B)	Cold Water Condenser (C)	Freeze Trap Following (D)
35%	100	114 ± 2	111 ± 1	95 ± 1
55%	100	113 ± 4	108 ± 2	90 ± 9
90%	100	118 ± 3	107 ± 2	96 ± 4

(a) Control Water collected by freeze trap and by molecular sieve (both methods identical results). Arbitrarily set at 100%.

(b) Condensed water was collected from the humidity control equipment (10°C).

(c) Air was drawn past a cold water condenser (20°C) and then through

(d) a freeze trap.

± Standard deviation of replicate analysis

These examples show that the failure to consider and apply all of the appropriate experimental controls and a failure to take into account all of the possibilities of isotopic fractionation can easily cause errors of up to 30% in tritium research.

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